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Emerging contaminants affect the microbiome of water systems—strategies for their mitigation

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The presence of emerging contaminants (ECs) in the environment has been consistently recognized as a worldwide concern. ECs may be defined as chemicals or materials found in the environment at trace concentrations with potential, perceived, or real risk to the "One Health" trilogy (environment, human, and animal health). The main concern regarding pharmaceuticals and in particular antibiotics is the widespread dissemination of antimicrobial resistance. Nevertheless, non-antimicrobials also interact with microorganisms in both bulk phase and in biofilms. In fact, drugs not developed for antimicrobial chemotherapy can exert an antimicrobial action and, therefore, a selective pressure on microorganisms. This review aims to provide answers to questions typically ignored in epidemiological and environmental monitoring studies with a focus on water systems, particularly drinking water (DW): Do ECs exposure changes the behavior of environmental microorganisms? May non-antibiotic ECs affect tolerance to antimicrobials? Do ECs interfere with biofilm function? Are ECs-induced changes in microbial behavior of public health concern? Nowadays, the answers to these questions are still very limited. However, this study demonstrates that some ECs have significant effects in microbial behavior. The most studied ECs are pharmaceuticals, particularly antibiotics, carbamazepine and diclofenac. The pressure caused by antibiotic and other antimicrobial agents on the acquisition and spread of antibiotic resistance seems to be unquestionable. However, regarding the effects of ECs on the development and behavior of biofilms, the conclusions of different studies are still controversial. The dissimilar findings propose that standardized tests are needed for an accurate assessment on the effects of ECs in the microbiome of water systems. The variability of experimental conditions, combined with the presence of mixtures of ECs as well as the lack of information about the effects of non-pharmaceutical ECs constitute the main challenge to be overcome in order to improve ECs prioritization.

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INTRODUCTION

The threat of emerging contaminants (ECs) or contaminants of emerging concern for the environment and human health is far from being understood[1,2]. Among huge varieties of ECs, several classes are highlighted: pharmaceuticals and personal care products (PPCPs), nanomaterials, fire retardants, pesticides, plasticizers, and surfactants[2]. More recently, some contaminants resulting from the use of several chemicals, as disinfection by-products (DBPs), and antibiotic resistant bacteria (ARB) and genes (ARG) have also been described as ECs by Berendonk et al.[3] and Richardson and Ternes[4]. The worldwide routined use of ECs is a result of economic growth and of increase in lifestyle patterns. It is expected that contamination by ECs will intensify due to the increase of global human population mainly in high-density areas, resulting not only in an increase in the ECs levels but also in the number of ecosystems contaminated. In developed countries the use of ECs is increasing and a putative reduction is an almost impossible task as they are absolutely indispensable for health and general life quality[5]. Despite the increased use of ECs and the consequent higher level of contamination in the environment, the impact from bioaccumulation and biomagnification should not be forgotten[6]. These are two important concepts that amplify the presence and the exposure to several contaminants that are able to accumulate in some organisms or tissues and that are propagated through the food chain. The concern about bioaccumulation and biomagnification of ECs in the environment emerged with the chlorinated hydrocarbons, mainly with the pesticide di-chlorodiphenyltrichloroethane (DDT) in the 1940s–1950s[2]. DDT was the first worrying example of a contaminant with huge impact in the environment and in the human health[2].

ECs may reach the environment through different routes[7,8] (Fig. 1), and wastewater treatment plants (WWTPs) are the main point of ECs entrance into the environment, mainly in surface waters[8]. The removal of ECs during water treatment in WWTPs and in drinking water treatment plants (DWTPs) is not effective[9]. Therefore, advances in analytical chemistry already allowed the detection of trace concentrations of ECs in different environments: surface water, groundwater, DW, swimming pool, soils, sediments, and irrigation water[10–16]. As a consequence, inhabitant organisms (i.e., algae, microorganisms, aquatic animals, vegetables, and humans) are continuously exposed to ECs (Fig. 1), which may cause direct or indirect consequences for each class of organism. The effects from such exposure are still not completely understood. For instance, the main concern regarding pharmaceuticals and in particular antibiotics is the widespread of antibiotic resistance in microorganisms[16,17], representing indirect consequences for other inhabitant organisms. In particular, DW microbiomes have been poorly studied. Although DW is considered microbiologically safe for consumption, based on the presence/absence of fecal contamination in the transported water, 95% of microorganisms in DWDS are attached on pipe walls as biofilms[18]. Biofilms constitute a significant problem for DW

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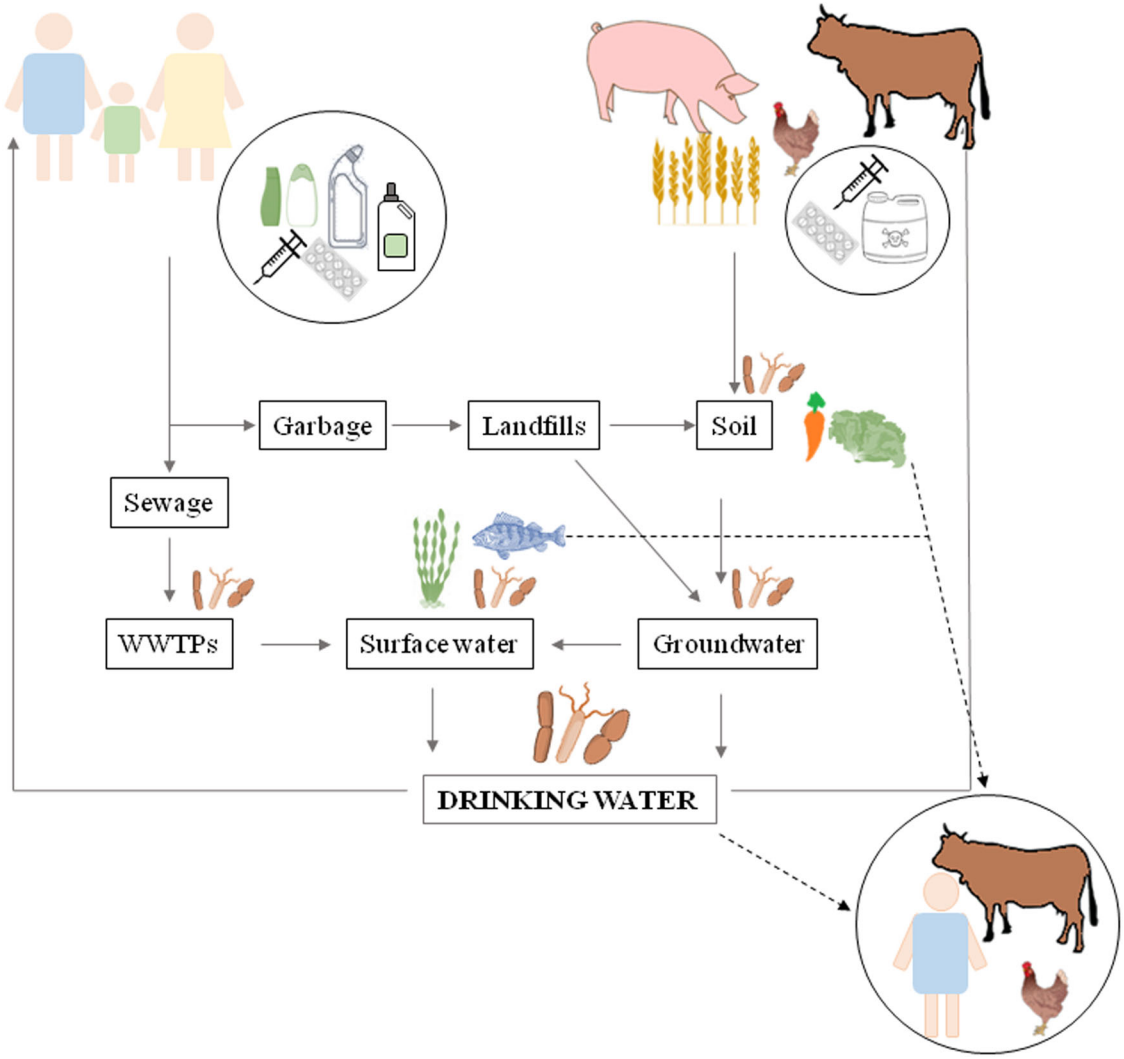

Fig. 1 Routes of ECs spread into environmental ecosystems and representation of organism continuously exposed to them. ECs reach different ecosystems through different routes, i.e., animal excretion, direct application and entrance in the sewage, direct application or disposal in soils. The inefficiency of water treatments in ECs removals makes WWTP one of the main sources of ECs into the environment. Different organisms (animals, vegetation, and microorganisms) are continuously exposed to ECs in the most varied ecosystems[7,8].

companies—beyond accelerating pipe corrosion and causing organoleptic changes in the delivered water, they may harbor environmental pathogens (e.g., *Legionella pneumophila*, *Pseudomonas aeruginosa*, and nontubercolosis *Mycobacterium*)[19]. The proliferation of environmental pathogens in biofilms increases health risks for DW consumers. For example, in 2014 most of the hospitalizations and deaths resulting from DW infections were related to environmental pathogens associated with biofilms in plumbing systems, resulting in an annual cost of US$ 2 billion[20]. Furthermore, microorganisms in DW biofilms are able to resist to unfavorable environmental conditions, such as nutrient starvation or the presence of residual disinfectants in the DW distribution systems (DWDS). For that reason, most of microorganisms present in DW are in a dormant state, an important survival strategy[21]. The exposure to all these stress conditions may alter the microbiome behavior to ECs. Factors affecting the presence of environmental pathogens in DWDS and plumbing systems are not yet well-known. Therefore, the possible effects from a continuous exposure to ECs on microbial virulence and pathogenicity, and consequently on the public health should be conveniently explored.

This literature review aims to present evidences on the microbiological role from ECs' presence in water bodies, with main emphasis in DW. This assembles the known data on the effects from the exposure to ECs in microorganisms naturally present in water bodies, including DWDS and reports the possible consequences for public health in terms of tolerance to antimicrobials and the dissemination of antibiotic resistance genes (ARGs) and ARBs. This information is of utmost importance in order to prioritize ECs according to the putative effects for public health and antibiotic resistance dissemination. Two strategies to reduced DW microbiome exposure to ECs are also reviewed.

PRESENCE OF ECS IN DW

ECs from the most diverse categories (PPCPs, nanomaterials, fire retardants, pesticides, plasticizers, surfactants, DBPs, ARB, and ARGs) have been detected in DW (Supplementary Table 1). The information available in Supplementary Table 1 shows that the most commonly detected are antibiotics (22/154, i.e., 14%), DBPs (11/154, i.e., 7%), perfluorinated compounds (16/154, i.e., 10%), pesticides (14/154, i.e., 9%), hormones (9/154, i.e., 6%), anti-inflammatory drugs (7/154, i.e., 4.5%) and UV filters (7/154, i.e., 4.5%). Carbamazepine was the EC most frequently detected and

mentioned in the literature (Supplementary Table 1). ECs reach DW due to the inability of DWTPs to completely remove these contaminants. Also, conventional WWTPs are not prepared to remove ECs from received water causing the dissemination of untreated ECs to surface waters or soils (through the reuse of activated sludge as fertilizer)[22]. Nevertheless, there are several works demonstrating that WWTPs may have important impact on the reduction of levels of specific ECs by biodegradation using activated sludge, adsorption to the activated sludge, by reaction with oxidative disinfectants as chlorine or ozone, or with ultraviolet (UV) treatment[22]. Taking into account that DW is often produced from surface water and groundwater, the efficacy of ECs removal in DWTPs is also important. Some conventional treatments in DWTPs, such as filtration, oxidation, and adsorption, may remove some of these contaminants. Several studies[9,23] demonstrated that oxidative processes, particularly the use of chlorine, are important strategies to reduce levels of ECs in DWTPs. The use of granular filters, such as sand, is also a common strategy applied in DWTPs with low ability to remove ECs[9,24]. The use of granular activated carbon is conventionally used in DWTPs with important results in the reduction of ECs content[9,23,24]. Despite all these barriers along conventional DWTPs, the removal of ECs is still inefficient and their presence in the final DW remains unavoidable[25]. The number of works studying the presence of ECs in DW have been increasing and the main works published in the last 10 years are listed in Supplementary Table 1, where it is possible to observe that chemically diverse ECs have been detected in DW all over the world.

There are multiple treatments applied in DWTPs and along DWDS that also aim to reduce the microbial load in the delivered DW. However, DW is not sterile and the development of biofilms along DWDS pipes is unavoidable. For that reason, microbial biofilms are continuously exposed to ECs in DWDS. Bacteria are the microorganisms more abundant in DW biofilms, but other organisms and biological structures are also found in DW biofilms, including viruses, protozoa, fungi, and algae[26]. The presence of ECs may have consequences on the behavior of DW microorganisms, particularly on the community diversity and function and on the spread of antibiotic resistance. The information regarding the consequences of ECs on the DW microbiome is scarce. Therefore, research on this topic is emerging in order to understand putative effects on the DW microbiome from ECs exposure and to develop strategies to reduce microbial exposure to ECs.

ECS IMPACT ON THE WATER MICROBIOTA

Conventionally, the screening of potential ecological risks of ECs is performed according to PBT (persistence, bioaccumulation and toxicity) criteria[5]. However, these criteria have demonstrated that some ECs, namely PPCPs only have toxic effects at high concentrations (higher than therapeutic doses). Therefore, the use of PBT criteria may lead to the conclusion that many ECs are not dangerous for the environment based on the fact that the concentrations detected in water and soil are very low (ng/L or µg/L)[5]. However, environmentally relevant concentrations (ng/L or µg/L) can alter ecological interactions[27–30]. Studying the effects of trace levels of ECs on the biota is relevant together with the definition of new criteria to assess the problems caused by ECs in the environment, even at trace concentrations. Several studies described alterations in animal behavior due to the exposure to some ECs[31,32]. For example, tadpoles (*Bufo arabicus)* exposed to fluoxetine (at 3 µg/L) were more susceptible to predation from dragonfly larvae (*Anax imperator*)[31]. Brodin et al.[32] described changes in the behavior of European perch (increased activity—number of swimming bouts for 10 min; increased boldness—the inverse of latency to enter a novel area during the total trial time; reduce sociality—cumulative time (in seconds) spent close to a group of co-specifics and reduced feeding rate) after exposure to oxazepan. Many other works have described the effects of ECs at trace concentrations in animal behavior[33–35]. ECs are also known to alter microbial communities and function, and may be responsible for spreading antibiotic resistance[36,37]. Studies have described the effects of different ECs exposure on the microbiota of water-related environments (rivers, marine environment, wetlands, etc), soil and in engineered systems (e.g., activated sludge from WWTPs)[36,38,39]. For example, Proia et al.[36] evaluated the effects of pharmaceuticals and pesticides on fluvial biofilms in a Mediterranean river. They observed an increase in autotrophic biomass and in peptidase, and a decrease of phosphatase and photosynthetic efficiency, when biofilms were moved to more polluted areas, i.e., with higher concentration of ECs. Ma et al.[38] studied the effects of oxytetracycline on the activity of soil microorganisms, simulating the application of oxytetracycline to soil in sewage sludge or manure or wastewater irrigation. The single addition of OCT caused an increase in microbial biomass carbon, and in the nitrification potential of the microbiota. Dehydrogenase activity sharply increased after 14 days, but decreased after 120 days. Daily application of OCT increased the McIntosh Index. Regarding the ECs effects on activated sludge, Kraigher et al.[39] assessed the impact of some pharmaceuticals on bacterial community structure in activated sludge from a small-scale wastewater treatment reactor. They observed a shift in the bacterial community in the reactor supplemented with pharmaceuticals at 50 µg/L. Information about the effects on DW microbiota is, however, still very scarce[40]. The main alterations caused by ECs in the water microbiota may be divided essentially in two main groups: microbial ecotoxicology and spread of antibiotic resistance.

Microbial ecotoxicology of ECs

Microbial ecotoxicology is an emerging topic that focuses on the ecological impacts of chemical or biological pollution on microbial diversity and on its functions in the stabilization and the recovery of ecosystems[41]. Although ECs have been detected in the most diverse environmental ecosystems, they are not included in routine monitoring programs and their ecotoxicological effects remains to be understood[42]. Natural microbiota of rivers, marine environments, wetlands, and even soils have important role on the equilibrium of ecosystem. The exposure to ECs, even at trace levels, may alter the diversity of the microbial community as well as their function in the ecosystem[27–30]. These alterations may influence the ecosystem function and biodiversity[43,44].

There are a number of studies describing the effects of emerging contamination on the behavior of natural microbiomes in different water sources, such as on rivers biofilms, in marine sediment biofilms and other aquatic ecosystems. Exposure to environmental relevant concentrations of ECs caused significant alterations in the microbial community composition and function, such as alterations in respiration rate, extracellular polymeric substances (EPS) production and enzymatic activity[40,45]. It is important to take into account that ECs are not found isolated in the environment, but in complexes mixtures of contaminants[10,14,46]. The mixture of ECs can have additive (i.e., response to multiple ECs is equal to the sum of their individual effects) or multiplicative ecotoxicological effects (i.e., the response exceeds the sum of their individual effects)[42]. Multiplicative interactions can be synergic (having a positive feedback) or antagonic (having a negative feedback)[47].

Effect of ECs on environmental biofilms. Aquatic biofilms play a crucial role in water purification as they actively participate in the removal of organic matter and nutrients, and have an important action in the biogeochemical cycle[44]. Biofilms in aquatic environments are generally composed by diverse microorganisms entrapped in a matrix of EPS produced by their own. The EPS

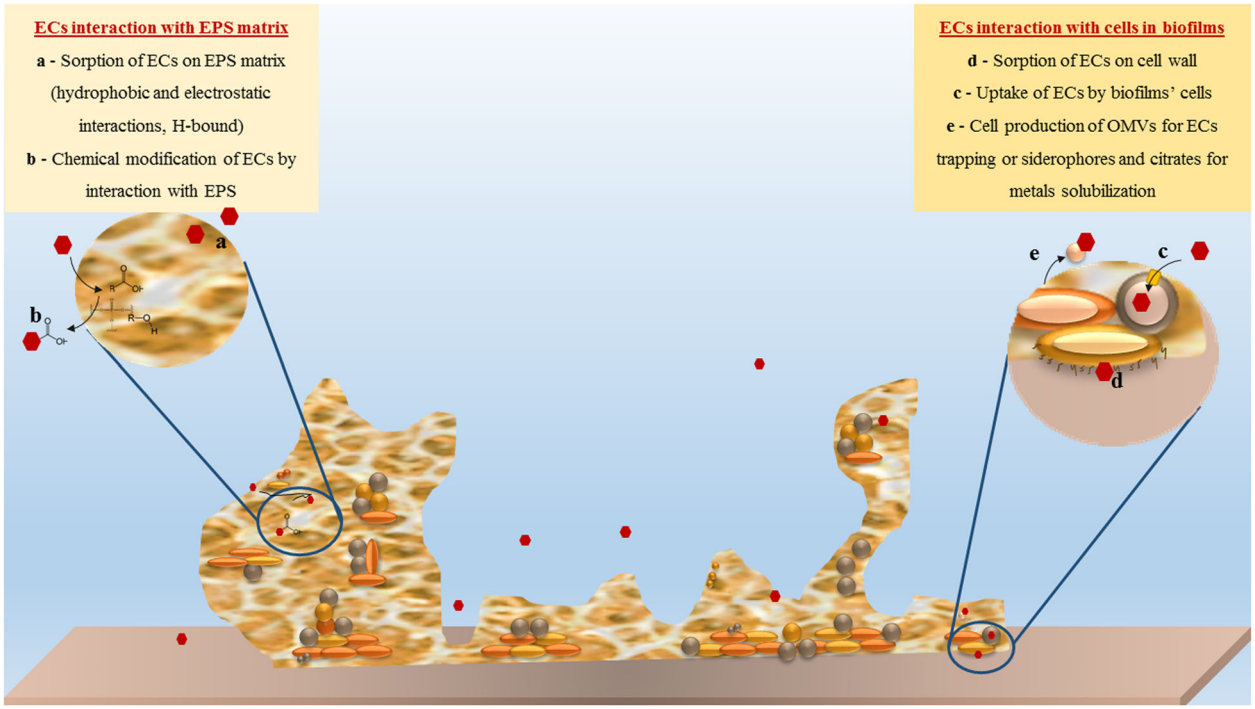

Fig. 2 Putative routes of interaction between ECs and biofilms. ECs may be uptaken by microbial cells in biofilms and can also adsorb to bacterial surface. Bacteria may produce vesicles and other compounds that may trap and/or solubilize ECs. EPS matrix also plays an important role on EPS adsorption and in its chemical modification. ⬢—emerging contaminants (ECs)[51–56].

matrix, mainly composed by polysaccharides, proteins, lipids, and nucleic acids, plays an important role in the sorption of contaminants[48]. In addition, aquatic biofilms can be used as indicators of the overall water quality[49], and have been used in ecotoxicological studies to evaluate the impact of multiple stressors in the ecosystem[50]. Biofilms have the ability to interact with environmental contaminants in multiple ways (Fig. 2). Bacteria in biofilm may uptake ECs from water. ECs may also be adsorbed in the bacterial surface by binding to specific sites, before being internalized[51–53]. Bacteria in biofilms may also produce siderophores and citrates that can complex with or solubilize some toxic metals[53]. The secretion of outer membrane vesicles from biofilm cells has also an important role in trapping some contaminants, such as antibiotics[54]. Moreover, ECs may also interact with the EPS matrix through adsorption to EPS (mainly by hydrophobic interactions between aliphatic and aromatic groups, electrostatic interaction between positively and negatively charged groups and the formation of hydrogen bounds)[55] or by chemical interactions with EPS-associated functional groups like (carboxyl, amine, hydroxyl, and phosphoric groups)[56].

A number of studies aimed to understand the effects of ECs on aquatic biofilms[27,29,57,58]. Most of them emphasize the effects of ECs on the microbial composition and metabolic function of biofilms in the ecosystem. Data on the effects of ECs in EPS production and composition is scarce. A significant part of these works used artificial streams inoculated with microorganisms collected from natural streams to form biofilms and to expose them to selected ECs[29,42,45,57,59,60]. Pharmaceuticals have been the ECs more studied[42,45,59,61], and less importance has been given to the effects of personal care products, plasticizers, perfluorinated compounds, pesticides, and other worrying ECs commonly found in water sources. Some works reported the effects of NPs in microbial behavior[62–66], however, most of these studies did not focus on biofilms but on ecotoxicological tests using planktonic microorganisms[64–66]. Supplementary Table 2 summarizes some works that evaluated the effects of ECs on the microbiome of water systems, reporting the exposure conditions and the main effects observed. Although the significant studies published about the effect of ECs on the microbiome of water systems, information about the effects of non-pharmaceuticals ECs (commonly found in environment and in DWDs—Supplementary Table 1) is lacking. There are several classes of pollutants often detected in surface waters worldwide[7] that have a recognized impact on aquatic fauna and flora, such as artificial sweeteners[67,68], personal care products[69,70], flame retardants[71], perfluorinated compounds[72], organic solvents[73], and complexing agents[74], whose effects on aquatic microbiome behavior have been disregard.

Regarding the variety of parameters studied to assess the consequences from ECs exposure on environmental microbiomes (Supplementary Table 2), it is possible to highlight the lack of standard tests to understand if the effects on the aquatic microbiome have significant impact in the stability of the ecosystem, i.e., concentrations of organic matter and nutrients, the biogeochemical cycle and/or food web.

Effect of ECs in DW biofilms. The effects of non-antibiotic ECs in DW at environmental levels (ng/L or µg/L) requires significant efforts to respond to environmental and public health concerns. We are aware of only four recent studies on this topic[40,75–77]. Wang et al.[40] evaluated the response of DW biofilms to sulfadiazine and ciprofloxacin. Gomes et al.[76] studied the effects from the exposure to different contaminants (antipyrine, caffeine, carbamazepine, clofibric acid, diclofenac sodium salt, galaxolide, ibuprofen, tonalide, tylosin, and trimethoprim—sulfamethoxazole) on the behavior of *Burkoldheria cepacia*, isolated from a DWDS in Portugal. Gomes et al.[75] focused their work on the effects of non-antibiotic contaminants on the behavior of *Stenotrophomonas maltophilia* isolated from DW. In another study, Gomes et al.[77] formed *S. maltophilia* biofilms for 12 weeks in the presence of clofibric acid and evaluated the susceptibility to antibiotics and chlorine, the effects in the production of virulence factors as well as in the bacterial ability to adhere and invade host cells (HT29

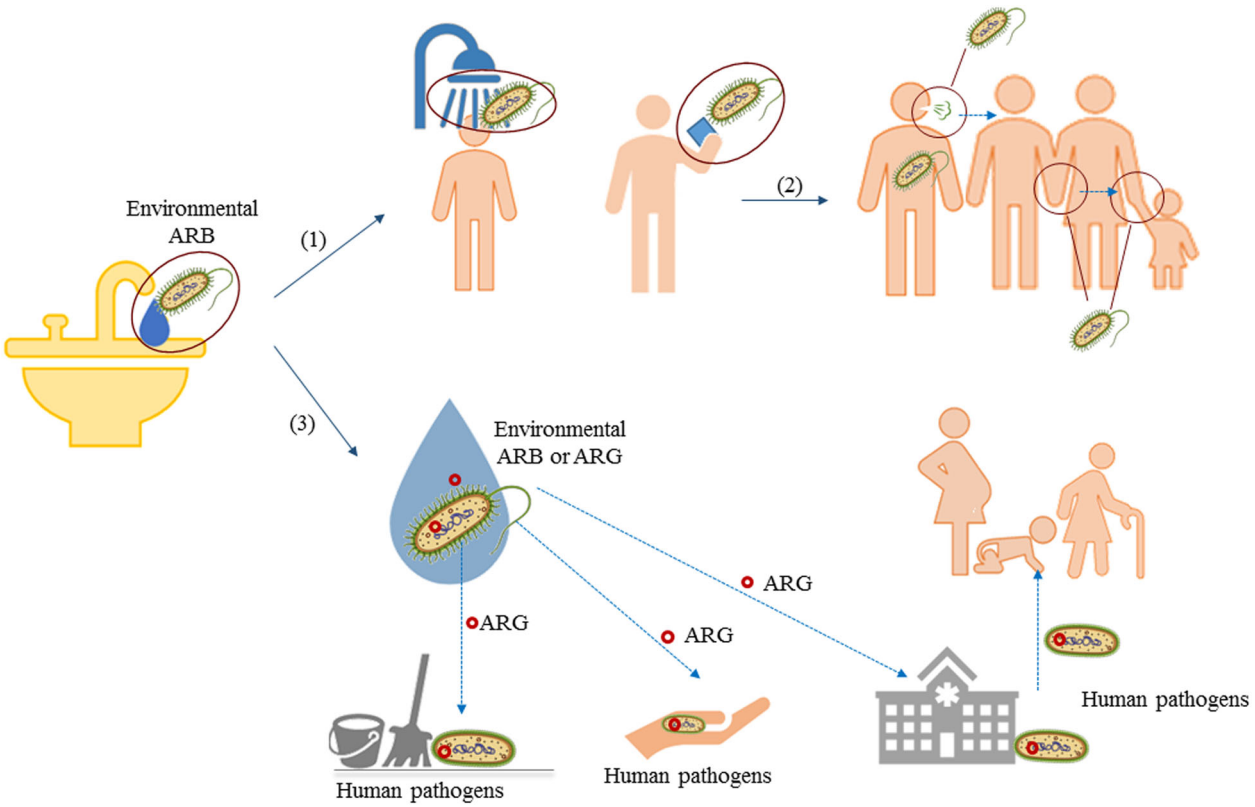

Fig. 3 Conceptual presentation on how DW antibiotic resistant elements (ARB and ARG) may threat human health. (1) human contamination with an antibiotic resistant pathogen through direct use/ingestion of water (2) human to human transmission after a direct infection with contaminated DW, for example, by skin contact, air, and saliva particles (i.e., sneeze); (3) horizontal ARG transfer to human pathogens through the contact of environment ARB and ARG with human pathogens present in the most varied environments, such as in surfaces of different buildings (floor, furniture, etc.), in hands and mainly in healthcare units, infecting mainly immunocompromised groups[83].

human cells from colorectal adenocarcinoma). Supplementary Table 3 summarizes the main findings described by the above mentioned studies. However, only a small portion (~8%) of the more than 150 ECs detected in DW mentioned in Supplementary Table 1 have been assessed in DW microbiome.

Most of the existing literature is focused on the effects of ECs on the behavior of specific bacteria isolated from DW. Only one study described the effects of two antibiotics on natural DW biofilms. Thus, the impact of ECs on DW biofilms remains to be understood. There is thus a need to study the effects of a wide range of antibiotic and non-antibiotic ECs on natural DW biofilms in order to improve the prioritization criteria for the development of adequate strategies for ECs treatment.

ECs and tolerance to antimicrobials

It is important to differentiate microbial resistance and microbial tolerance to antimicrobial agents. Microbial tolerance may be defined as the ability (genetic or not) of microorganism to survive from the exposure to high concentrations of antimicrobials. On its turn, microbial resistance is generally associated with inherited mutations[78]. ARB and ARG occur in nature and are already considered contaminants of emerging concern. The mechanisms responsible for bacterial resistance to antimicrobials are inherent in part to the fast changes of the bacterial genome. Bacterial genome alterations are not only a consequence of mutations or genome rearrangements during bacterial life cycle, or as a result of selective pressure, but it may also be related to the acquisition of exogenous genes through the exchange between microorganisms or by the gene capture in integrons through horizontal gene transfer[17,79]. Integrons are natural gene expression systems that can act as reservoir of silent genes mobilizable when necessary. That mobilization by site-specific recombination transform silent genes in functional genes[79].

The presence of ARG and ARBs in different ecosystems worldwide is of particular concern[80]. DW is recognized as a reservoir of ARB and ARG and it is known that the abundance of ARB and ARG is higher in tap water than in finished water (in the outlet from DWTP)[81,82]. Antibiotic resistance in DW may threaten human health in three different ways (Fig. 3): (1) human may be contaminated with an antibiotic resistant pathogen through direct ingestion of water; although no human to human transmission occurs; (2) human to human transmission occurs after a direct infection by consumption of contaminated DW; and (3) horizontal ARG transfer to human pathogens[83]. There are only few studies reporting the occurrence of outbreaks of diseases due to the presence of ARB in DW[83–85]. The spread of antibiotic resistance is linked to the use of antibiotics. Qiu, et al.[58] analyzed 31 water and sediment samples from rivers in China and observed positive correlations among *blad* gene, *Fusobacteria* and sulfamethoxazole. The authors suggested that antibiotic exposure may be positively linked to the expression of ARG in certain bacteria[58]. Wang et al.[40] reported significant effects from antibiotic exposure on the spread of ARG in DW biofilms. The exposure to sulfadiazine and ciprofloxacin enhanced the abundance of resistance genes for these antibiotics. Exposure to ciprofloxacin also induced *mexA* expression, a gene encoding for an efflux pump conferring bacterial resistance to antibiotics. Exposure to ciprofloxacin and sulfadiazine simultaneously had higher impact in the abundance of *mexA* and *intI1* (class I integrase gene)[40]. Nevertheless, bacteria in the environment are exposed to mixtures of contaminants (pharmaceuticals and non-pharmaceuticals) and have to develop mechanisms enabling toleration[17]. In particular, the importance of

other non-antibiotic chemicals in the spread of ARG may have been overlooked[17]—there are some studies relating the exposure to diverse non-antibiotic contaminants with the development of antibiotic resistance in environmental microbiomes. Wang et al.[28] found that the exposure of coastal water microbiome to polycyclic aromatic hydrocarbons accelerated the propagation of ARGs. These authors reported that naphthalene at 100 mg/L and phenanthrene at 10 mg/L enhanced the abundance of *intI1*, *sul1* (sulfanilamide resistance gene), and *aadA2* (aminoglycosides resistance genes) in coastal microbiome. *Intl1* and *sul1* were also induced in stream biofilms after exposure to a mixture of different antibiotic and non-antibiotic ECs (ciprofloxacin, erythromycin, sulfamethoxazole, diclofenac, and methylparaben)[27]. The availability of high concentrations of nutrients favored the spread of the referred ARGs when the microbial community was also exposed to the cocktail of ECs[27]. Lv et al.[86] evaluated the effects of DBPs on the development of antibiotic resistance in *Pseudomonas aeruginosa* PAO1. The exposure of *P. aeruginosa* to bromoacetamide, trichloroacetonitrile or to tribromonitromethane increased resistance to ciprofloxacin, gentamycin, polymyxin B, rifampin, tetracycline, ciprofloxacin + gentamicin, and ciprofloxacin + tetracycline. These authors demonstrated that alterations in antibiotic resistance were caused by mutagenesis, associated with an overexpression of efflux pumps[86]. On the other hand, several other works also reported that some non-antibiotic ECs promote horizontal gene transfer intra and across bacterial genera. Some examples are triclosan[87], carbamazepine[37], disinfectants, such as free chlorine, chloramine, and hydrogen peroxide[88], and metals (copper, silver, chromium, and zinc)[89].

Although the existence of several studies evaluating the effects of ECs on ARG and ARB occurrence in water systems, the effects of ECs on the spread of antibiotic resistance and consequently the risk for public health have not been explored in the literature. It is also important to take into account that alterations in the bacterial genome may be drove by different environmental factors, such as alterations in pH, temperature, nutrient availability, and presence of substances foreign to cells. Therefore, the effect of a specific substance on the increase of ARG or ARB bacteria in a specific water source (i.e., surface water with higher availability of nutrients) will not be necessarily the same effect caused in another water source (i.e., DW—an oligotrophic environment in the presence of residual disinfectants).

TECHNICAL APPROACHES TO REDUCE THE EMISSION OF ECS AND THEIR INTERACTION WITH DW BIOFILMS

Effective measures to reduce the exposure of natural microbiomes to ECs are needed. Special attention should be given to DW microbiome. Two strategies can be followed to reduce the exposure of microbial communities to ECs: (1) reduce the entrance of ECs in DWDS and (2) prevent biofilm development in DWDS. However, it is important to take into account that there are no strategies able to eradicate biofilms in DWDS and the complete removal of ECs from DW is also not feasible.

There are several treatments that can remove partially or completely some ECs. Nevertheless, the presence of complex mixtures of ECs with different physical and chemical properties in the environment highlights the complete removal of ECs as challenging.

DWTPs are composed by multi-barrier processes to produce safe DW. The processes, include oxidation (chlorination, ozonation and other advanced oxidation processes (AOPs)), filtration (including biofiltration), coagulation, flocculation, settling, and also secondary disinfection[90,91]. However, conventional DWTPs are not effective in the removal of ECs and the concentration of these contaminants remains relatively unchanged in the finished DW[12].

Coagulation, flocculation, and settling

Coagulation and flocculation are not very effective in the removal of ECs. These processes are commonly designed to remove suspended solids. Therefore, ECs can only be removed if they are able to adsorb on the flocs formed[92]. Boiteux et al.[93] and Sun et al.[94] evaluated conventional DW treatments on the removal of PFAS (per- and polyfluoroalkyl substances) and observed that coagulation followed by sedimentation did not cause PFAS removal. Flocculation using iron (II) chloride was unable to eliminate bezafibrate, clofibric acid, carbamazepine, and diclofenac[95]. Petrovic et al.[96] also evaluated the efficiency of different processes on the removal of estrogenic short-chain ethoxy nonylphenolic compounds and found that settling, flocculation and sand filtration processes only removed 7% of the ECs present in water. On its turn, Westerhoff et al.[97] evaluated the removal of 62 different contaminants (endrocine disruptor compounds and PPCPs). The use of aluminum sulfate and ferric chloride coagulants removed some polyaromatic hydrocarbons, with removal rates lower than 25%[97]. More recently, Su et al.[98] investigated the occurrence and diversity of ARGs in DW treatment processes and observed that sedimentation is an important process able to effectively reduce the abundance of ARGs.

Filtration processes

Filtration is an important process in DWTP and can be divided into two main applications: (i) rapid filtration processes—aiming to separate solid particles in water and that usually represent the last clarification process following processes like coagulation, flocculation or sedimentation processes; and (ii) slow filtration process—with filters with smaller grains and consequently lower pore size, improving general water quality even in terms of microbial content[99]. There are several filter media commonly used such as sand, activated carbon and anthracite, and the removal of contaminants will be driven by the pore size and the adsorption to the selected media. There are several works evaluating the ability of conventional filtration (sand, activated carbon, and anthracite) on the removal of different ECs[92,97,100,101]. Sand filtration is not effective in the removal of ECs[93,95,100,101], but may have important effects on the reduction of ARGs abundance in DW[98]. On the other hand, activated carbon have a more variable performance, as represented in Table 1, since it only removes dissolved ECs able to adsorb on its surface. Activated carbon efficiency on ECs removal is dependent of several factors: water matrix, type of carbon used, activated carbon usage rate and loading[100]. ECs octanol-water partition coefficient may drive their adsorption to activated carbon[92,97]. Boiteux et al.[93] evaluated the removal of PFAS in three DWTPs and observed that activated carbon filtration was inefficient in PFAS removal. Moreover, the use of GAC in DWTP seems to have a negative impact in the removal of ARGs[98]. Anthracite is also a common filter matrix applied in DWTP able to significantly reduce the concentration of estrogens, removing 84–99% of these ECs[101].

Biofiltration consists in the development of biofilms in media filters surface (sand, activated carbon, etc). This process has also been studied in terms of ability to remove ECs in DWTPs[94,102–104], as described in Table 1.

Membrane filtration processes are advanced treatments that have significant impact in DWTPs, including in the improvement of ECs removal. These methods employ a semipermeable membrane to separate materials according to their physical and chemical properties, through pressure differential or electrical potential difference[99,104]. Nanofiltration (NF), UF, and reverse osmosis (RO) are the most commonly applied in DWTPs and their performance has been important in the removal of different ECs[93,100,105]. NF is an important strategy to remove PFAS from water[93] as well as RO (>99% removal)[100]. UF demonstrated to be a non-efficient process to remove PFAS[100]. Yoon et al.[105] studied the

Table 1. ECs removal efficiency in DWTPs by different filtration processes.

Processes	ECs	Removal obtained	Ref.
Activated carbon	ARG	0.01 log (PAC)/−0.15 to −0.21 log (GAC)	98
	Benzafibrate	>70 m^3/kg (specific throughput)	95
	Bisphenol A	80% (GAC)	95,102
	Carbamazepine	71–93% (GAC)/50–70 m^3/kg (specific throughput)	
	Clofibric Acid	10–17 m^3/kg (specific throughput)	
	Diclofenac	>70 m^3/kg (specific throughput)	
	Ethoxy-Nonyl Phenolic	73% (GAC)	96
	Gemfibrozil	44–55% (GAC)	102
	PFOA	45% (GAC)	100
	PFOS	64% (GAC)	
	Polar Contaminants	10–95% (PAC)	97
	Volatile Contaminnats	50–98% (PAC)	
Anthracite	Estrogens	84–99%	96
Sand	ARG	0.35–0.48 log	98
Biofiltration	17β Estradiol	>80% (GAC)	104
	2,4-Dichlorophenoxyacetic Acid	68–77 % (sand)	103
	2-Methylisoborneol	93–99% (sand)	
	Acetochlor	8–17% (sand)	
	Acetominophen	59–79% (sand)/ > 80 % (GAC)	103,104
	Aldicarb	49–72% (sand)	103
	Aminotriazole	<30 % (GAC)	104
	Atenolol	>75% (GAC)	104
	Atrazine	0.2 - 3% (sand)/ < 30 % (GAC)	103,104
	Bisphenol A	64% (sand)	103,104
	Caffeine	67–80% (sand)	
	Carbamazepine	0.5–1.6% (sand)/<30% (GAC)	
	Carbaryl	3.3–17% (sand)	103
	Chlorpyrifos	63–83% (sand)	
	Clofibric Acid	35–52% (sand)	
	Cotinine	23–39% (sand)/<30% (GAC)	103,104
	DEET	<30% (GAC)	
	Diazinon	12–40% (sand)	103
	Diclofenac	>80% (GAC)/21–28% (sand)	102,103
	Dimethoate	75–81 % (sand)	103
	Diuron	0.3–7.8% (sand)	
	Erythromycin	15–27% (sand)	
	Ethinyl Estradiol	12–22% (sand)	
	Gemfibrozil	70–94% (sand)	
	Ibuprofen	>80% (GAC)/>95% (sand)/>75% (GAC)	102–104
	Iopromide	3–13% (sand)/<30% (GAC)	103,104
	Malaoxon	16–49% (sand)	103
	Methomyl	5.3–12 % (sand)	
	Metolachlor	6.6–8.7% (sand)	
	Molinate	85–97% (sand)	
	Naproxen	>80% (GAC)/72–86% (sand)	102,103
	Prometon	0–2.5% (sand)	103
	Simazine	6.8–8.2% (sand)	
	Sulfamethoxazole	2.4–4.1% (sand)	
	TCEP	<30% (GAC)	104
	Tributyl Phosphate	16–24% (sand)	103
	Triclosan	>90% (sand)	
	Trimethoprim	83–92% (sand)/>80% (GAC)	103,104
	Warfarin	39–68% (sand)	103

efficiency of NF and UF membranes on the removal of 52 ECs with different physicochemical properties (size, hydrophobicity, and polarity). NF was able to retain many ECs due to hydrophobic adsorption and due to size exclusion. On the other hand, UF retained only hydrophobic ECs due to hydrophobic adsorption to membrane[105].

To date, the available literature demonstrated the limited efficiency of settling and conventional filtration processes on the

removal of ECs. Yang et al.[106] evaluated the removal of eight phthalate esters and thirteen pharmaceuticals from DW using a complex system of simultaneous electrocoagulation and electrofiltration (EC/EF) based on a tubular carbon nanofiber/carbon/alumina composite membrane for DW filtration. The application of an electric field improved the removal of phthalate esters from 20 to 45% (without electric field) up to 78% and the pharmaceuticals up to 77%. In the conventional systems the removal was caused by steric exclusion based on the pore size/network microstructure and due to the adsorption to carbon nanotubes. However, the application of an electric field reduces the formation of filter cake on the membrane surface, improving the permeate flux, and thus removal efficiencies. The overall disadvantage of these physical methods is the expensive treatment of the contaminated adsorbent material and of the waste generated[107].

Conventional and advanced oxidation processes

Oxidation processes are of utmost importance in DW treatment for the control of microbial load. Conventional oxidation processes use strong oxidant reagents to inactivate microorganisms, such as free chlorine, chlorine dioxide, chloramines, hydrogen peroxide, and ozone. Nevertheless, the reaction of these oxidants with organic matter can form carcinogenic halogenated DBPs. AOPs emerged as an important technology for DW treatment without the formation of DBPs[108].

The oxidation power of conventional and advanced processes is very important in the degradation of ECs in DWTPs[92,93,96,100,109,110]. Chlorine was able to degrade some ECs such as methyl indole and in lower extent chlorophene and nortriptyline[109]. Benzatriazole and N,N-diethyl-m-toluamide were recalcitrant and was not altered by chlorine[109]. Oxidation processes, such as chlorination, chlorine dioxide treatment, peroxidation, and ozonation were not able to remove PFAS in DWTPs[93,94,100] probably due to the strength of the C–F bond. Su et al.[98] also reported inefficiency of ozonation in DWTPs since the abundance of some ARGs increased after water treatment with ozone. Westerhoff et al.[97] reported that chlorine and ozone were able to degrade part of the ECs present in water and observed that ECs that are easily oxidized by chlorine are always oxidized at least as efficiently by ozone. Among the 62 contaminants analyzed only six were poorly oxidized by chlorine and ozone. In general, the use of ozone demonstrated potential to improve ECs removal[97]. Sichel et al.[110] reported the use of conventional (HOCl and ClO_2) and advanced (H_2O_2/UV, HOCl/UV, and ClO_2/UV) oxidation processes for DW treatment. Sulfamethoxazole was easily degraded by free chlorine and chlorine dioxide both at 6 mg/L and 15 min of contact. Benzatriazole, tolyltriazole, carbamazepine and iopamidole were not degraded by these processes. Chlorine-based AOPs completely removed 17α-ethinylestradiol and H_2O_2/UV caused significant removal of this contaminant. Sulfamethoxazole and diclofenac were readily degraded by all the AOPs evaluated. The higher removal efficiencies (40–60%) for desethylatrazine and carbamazepine were obtained through H_2O_2/UV. Removal yields of 60–80% and 80–100% for benzatriazole and iopamidole, respectively, have been reported with HOCl/UV. Benotti et al.[111] demonstrated that a membrane pilot system employing TiO_2/UV was able to reduce the concentration of 32 ECs including pharmaceuticals and endocrine disruptor compounds. This strategy caused removals superior to 70% for 29 of the evaluated ECs (for example, estrone, estradiol, ethinylestradiol, bisphenol A, octylphenol, butylated hydroxyanisole, atorvastatin, triclosan, diclofenac, sulfamethoxazole, and naproxen). The removal of the three remaining ECs (PFOS, tris(2-chloroethyl) phosphate, and tris(1-chloro-2-propyl) phosphate) was below 50%.

DWTPs are doted of all these referred barriers that aims to ensure the delivery of DW chemically and biologically safe. The previous Sections "Coagulation, flocculation and settling", "Filtration processes", and "Conventional and advanced oxidation processes" described several methods that may remove some ECs in DWTP, reducing their concentration in the final DW. Nevertheless, the higher challenge is to establish a compromise between the removal of the most worrying ECs and ensure the efficiency and economical viability of the processes. The presence of complex mixtures of ECs in raw water as well as the wide diversity of properties of these ECs may hinder the implementation of adequate measures. Therefore, it is important to prioritize ECs in order to understand whose ECs may constitute higher risks in each specific situation and them implement and/or improve the strategies most effective on the removal of the selected ECs. However, it is important to have in mind that the removal efficiency of each process will also dependent on the water characteristics.

Interaction with DW microbial communities

The microbial community colonizing DWDS is mainly present as biofilms—95% of the microbial community is adhered on surfaces[18]. Biofilms confer several advantages for microbial growth under conditions of low nutrient content and of environmental stress, as found in DWDS. The main advantages are related to the protection conferred by the EPS matrix that retains and stores nutrients and also binds to, and mop up some disinfectants. The EPS matrix also confers limitations to mass transfer avoiding and/or retarding the diffusion of disinfectants and other stressors through the matrix. Bacteria in biofilms have low metabolic rates and may acquire a dormant state, which can be an important strategy for their survival under stress conditions[26,112,113]. In addition, the development of biofilms in DWDS may be responsible for changes in the organoleptic characteristics of the delivered water[114]. It is also known that biofilms have impact in metal corrosion, accelerating the degradation of pipes and reducing their lifetime, and constitute a reservoir of microorganisms and a potential source of pathogens and ARGs[112]. Attending these microbial problems, DWTP comprises several processes aiming to guarantee the delivery of chemically and biologically safe water. All the processes referred previously (settling, oxidation, filtration, and secondary disinfection) have also an important action on the control of microorganisms and on the nutrient load of the finished water.

ECs may interact with microbial communities and biofilms in multiple ways (Fig. 2). Microbial cells may uptake ECs from water or release substances that will degrade/trap some ECs (biodegradation). The adsorption of ECs on the biofilm EPS matrix may also occur, reducing the ECs availability in bulk water. However, the literature regarding the consequences from the presence of ECs for DW biofilms is very limited, as discussed in Section "Effect of ECs in DW biofilms". On the other hand, the information about the interaction between the ECs and microbial communities of activated sludge in WWTPs is more abundant in the literature, which may be important to infer the possible effects of ECs in the DW microbiome. However, the variability of operating conditions between WWTP and DW cannot be disregard as the ECs–microbial community interactions will be influenced by nutrient availability, flow rate/intermittency, residence time, microbial community composition, pH, temperature, among others.

The activated sludge-based processes are designed mostly to remove the chemical oxygen demand, nutrients, and pathogens. However, it also constitutes the final defense system to avoid the entrance of ECs into water sources[115]. Nevertheless, the biodegradation of most of the ECs by activated sludge treatments is very limited, but the adsorption processes play a significant role in reducing the concentrations of ECs in WWTPs effluents. The presence of ECs may cause inhibitory or toxic effects to activated sludge microorganisms, reducing the efficiency of the biological treatment[116]. Some studies also reported the modification of activated sludge microbial community as well as their enzymatic activity due to the presence of some ECs[115]. Notwithstanding, in

some cases the treatment efficiency was not compromised by these alterations[117].

In order to improve the removal of ECs using activated sludge-based treatments, Wang and Wang[118] proposed the acclimation of microbial communities in activated sludge. The authors further emphasized the research needs on the potential of biomass acclimation in the ECs removal efficiency.

CONCLUSIONS

The interest on the evaluation of ECs' presence in the environment has increased in recent years. However, the knowledge on the possible consequences from ECs exposure to the intrinsic microbiome is still limited. The exposure to ECs, even if at trace levels, may alter the microbial diversity and their function in the ecosystem. These changes may have important impacts in the function and biodiversity of the ecosystem—the colonizing microorganisms are responsible for depuration and nutrient removal in rivers, and for the nutrient cycle and plant interactions in the soil. The information available on the effects of ECs on DW microbiome is scarce. In most of the works, the knowledge is limited to a few range of ECs and to biofilms formed only by one species of bacteria isolated from DW.

A reduction on the ECs by acting on the source is impossible taking into account the societal dependence on these compounds. Therefore, the development of efficient strategies to remove ECs is of utmost importance to impair putative risks for the public health. DWTPs are endowed of several strategies that may reduce levels of ECs in the treated water. The need of more complete information on the effects of ECs for DW microbiome is rising. This information will be crucial for the correct prioritization of ECs' removal in DWTPs and, consequently, to improve the strategies used for ECs treatments. This study demonstrates that some ECs have significant effect in microbial behavior, being pharmaceuticals, particularly antibiotics (ciprofloxacin, erythromycin, and sulfamethoxazole), carbamazepine and diclofenac the ECs typically studied in terms of effects in microbiomes of water systems. It seems unquestionable that antibiotics and other antimicrobial agents have a significant role on the spread of antibiotic resistance. However, the available literature is not so conclusive about the effects of ECs on biofilms development and behavior. The present data is controversial, proposing that standardized test should be developed for an accurate analysis of ECs effects. It is important not to disregard that the effects of ECs may depend on different factors: ECs concentration, nutrient availability, hydrodynamics, time of exposure, etc. The variability of experimental conditions, combined with the presence of mixtures of ECs as well as the lack of information about the effects of non-pharmaceutical ECs constitute the main challenges to be overcome for a "One Health" ECs prioritization.

DATA AVAILABILITY

The authors declare that all data supporting the findings of this study are available within the paper, in the references cited.

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AUTHOR CONTRIBUTIONS

I.B. Gomes collected the data, designed and wrote the work. J.-Y. Maillard substantively revised the work. L.C. Simões substantively revised the work. M. Simões designed and revised the work.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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